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Peer Review Report for "Evaluating the Effect of Hydroalcoholic Extract of *Tragopogon graminifolius* DC in Treatment of Polycystic Ovary Syndrome in Female Wistar Rats"

Author(s): Mohammad Mehdi Gravandi, Pershia Davoodi Karsalari, Samira Shirooie, Maedeh Tahvilian, Mohammad Hosein Farzaei

Review Timeline:

Submit Date:	16 Apr 2025
Revised Date:	3 Jul 2025
Accepted Date:	11 Jul 2025

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFeree : Reviewer

23 Apr 2025

- 1- In the text with reference 15, it is mentioned that it is about Persian medicine, but in the referenced article , Iranian medicine sources are not mentioned and most of the traditional uses are reviewed, not necessarily Persian medicine.
- 2- In the introduction, in diagnosing PCO, it is better to refer to the view found in the ultrasound.
- 3- Why was the insulin level not checked or was it possible to measure the insulin level?
- 4- Was the number of follicles in the ovaries also checked or were only the follicles checked for maturation?

REFEREE : Reviewer

1 May 2025

Evaluating the Effect of Hydroalcoholic Extract of *Tragopogon Graminifolius* DC in Treatment of Polycystic Ovary Syndrome in Female Wistar Rat

Recommendation: Major Revision

1. The introduction states that PCOS affects 5-10% of the population. Please update this statistic with more recent epidemiological data (citations post-2022).
2. Given your hypothesis that the hydroalcoholic extract of *T. graminifolius* exerts its beneficial effects in PCOS by modulating oxidative stress and hormonal imbalance, why did you not measure oxidative stress markers (such as MDA, SOD, GPx) and inflammatory cytokines (such as TNF-6) in this study?
3. Provide a specific citation or rationale for using a testosterone enanthate dosage of 1 mg per 100 g of body weight? Review the manuscript to ensure that all dosages are consistently formatted in mg/kg throughout the text.
4. Did you confirm the successful induction of PCOS in the animals before starting the treatment phase? How was PCOS confirmed?
5. Why was a metformin dosage of 20 mg/kg chosen? Is this a standard dose for rat studies? Provide a specific citation for it.
6. Please provide supporting evidence from preliminary data or literature for the selection of *T. graminifolius* extract doses (100 mg/kg and 200 mg/kg).
7. Provide the specific dose of sodium pentobarbital used for euthanasia and a reference to support it.
8. Please specify the catalog numbers and suppliers for each ELISA kit.
9. The document mentions using Turkey's multiple comparison procedure. Correct it to Tukey's multiple comparison procedure.
10. To enhance clarity and avoid confusion, I recommend using "PCOS group" instead of "untreated group" throughout the manuscript when referring to the group of animals with induced PCOS that did not receive treatment.
11. For the sentence, "The findings on testosterone levels are presented in Figure 1.

In the untreated group, testosterone levels showed a marked rise," please specify that this increase in testosterone levels in the PCOS group is statistically significant compared to the healthy control group and include the relevant p-value in the text.

12. Correct the unit for LH levels in Figure 2.

13. If figure legends incorrectly state that the PCOS group received saline via injection, revise them accordingly.

14. When a term is written out in full for the first time, it should be followed by its abbreviation in parentheses. After the first instance, only the abbreviated form should be used. Review the entire manuscript to ensure this rule is followed consistently for all abbreviations.

15. The descriptions of the figures are not clear enough. For example, in Figure 1, MET is defined as "Reference group given metformin at a daily dosage of 300 mg/kg by ingestion". The dosage appears to be incorrect because in the methods, the dosage of Metformin is "20 mg/kg body weight each day over a 28-day period." Please ensure that the figure legends are consistent.

16. Please provide more information as to what was observed at the microscopic level to arrive at the end observation. Were there specific scoring systems used to quantify follicular development, cystic changes, or other histological features? In addition, please provide the complete set of the histopathological images with clearer labels in Figure 5.

17. In the Discussion section, compare your findings with existing literature to provide context. Discuss how your results support or contradict previous studies.

REFEREE : Associate Editor

10 May 2025

Thank you for submitting your manuscript to JJNPP.

Please revise the manuscript and submit a point-by-point response to the reviewers' comments and concerns and highlight the changes in the text.

I would also greatly appreciate the authors contribution and time for reviewing manuscripts for this journal. Without the dedication of reviewers like you, it would be impossible to manage an efficient peer review process and maintain the high standards necessary for a successful journal.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Cover letter

Dear editorial Team of the JJNP journal,

We sincerely thank you for considering our manuscript titled “Evaluating the Effect of Hydroalcoholic Extract of *Tragopogon Graminifolius* DC in Treatment of Polycystic Ovary Syndrome in Female Wistar Rat” for potential publication in your esteemed journal.

In response to the reviewers’ and the associate editor’s valuable feedback, we have thoroughly revised the manuscript. All modifications have been clearly highlighted in the main text. Additionally, we have prepared a detailed point-by-point response to each comment, which we believe addresses the concerns raised with clarity and precision.

We would like to particularly thank the associate editor for coordinating the review process and for their constructive remarks. We hope the revised version of our manuscript meets the journal’s standards and the reviewers’ expectations.

We would also like to mention that the license for the graphical abstract containing identifying information (such as author names) is prepared and will be submitted as a separate file. To maintain integrity of the double-blind peer review process, we have not attached it here, but it will be available upon acceptance.

Thank you once again for the opportunity to improve our work through this review process.

Sincerely,

Dr. Mohammad Hossein Farzaei

(Corresponding author)

Reviewers' Comments:

Reviewer 1:

We thank Reviewer 1 for their insightful and constructive feedback. Below are our point-by-point responses:

1- In the text with reference 15, it is mentioned that it is about Persian medicine, but in the referenced article, Iranian medicine sources are not mentioned and most of the traditional uses are reviewed, not necessarily Persian medicine.

Response: We revised the text to refer to “traditional medicine” instead of “Persian medicine” to avoid confusion and ensure accuracy.

2- In the introduction, in diagnosing PCO, it is better to refer to the view found in the ultrasound.

Response: The role of ultrasonography in diagnosing polycystic ovaries has now been more clearly emphasized in the introduction. We elaborated on the typical ultrasound features, such as the presence of multiple small follicles and increased ovarian volume, which support the diagnosis of PCOS. The added sentence has been highlighted in the

updated manuscript.
3- <i>Why was the insulin level not checked or was it possible to measure the insulin level?</i> Response: due to the limitation of the amount of serum, the levels of fasting blood glucose levels were analyzed as the factor of insulin tolerance which occurs in PCOS.
4- <i>Was the number of follicles in the ovaries also checked or were only the follicles checked for maturation?</i> Response: The number of follicles were not checked but the maturation and the phase of follicles were checked.
<i>Reviewer 2:Evaluating the Effect of Hydroalcoholic Extract of Tragopogon Graminifolius DC in Treatment of Polycystic Ovary Syndrome in Female Wistar Rat</i> <i>Recommendation: Major Revision</i> We thank Reviewer 2 for their thorough review and valuable suggestions. We have addressed the points below to the best of our ability, and we hope the revisions meet your expectations:
1. <i>The introduction states that PCOS affects 5-10% of the population. Please update this statistic with more recent epidemiological data (citations post-2022).</i> Response: We have updated the prevalence data by incorporating two recent systematic reviews and meta-analyses published after 2022 to ensure accuracy and strengthen the reliability of the epidemiological context.
2. <i>Given your hypothesis that the hydroalcoholic extract of T. graminifolius exerts its beneficial effects in PCOS by modulating oxidative stress and hormonal imbalance, why did you not measure oxidative stress markers (such as MDA, SOD, GPx) and inflammatory cytokines (such as TNF-6) in this study?</i> Response: we thank the reviewer for this commend, we will do this and the other signaling pathways for the next study to ensure the mechanism of the extract.
3. <i>Provide a specific citation or rationale for using a testosterone enanthate dosage of 1 mg per 100 g of body weight? Review the manuscript to ensure that all dosages are consistently formatted in mg/kg throughout the text.</i> Response: A relevant citation has been added to justify the dosage used. We have also revised the manuscript to ensure all dosages are consistently expressed in mg/kg.
4. <i>Did you confirm the successful induction of PCOS in the animals before starting the treatment phase? How was PCOS confirmed?</i> Response: Based on previous studies that we have conducted at this center and published articles, this model leads to a very high percentage of changes related to PCOS, including hormonal and histological changes in the animal as following our related articles: - Shirooie S, Khaledi E, Dehpour AR, Noori T, Khazaei M, Sadeghi F, Sobarzo-

Sánchez E. The effect of dapsons in testosterone enanthate-induced polycystic ovary syndrome in rat. The Journal of steroid biochemistry and molecular biology. 2021 Nov 1;214:105977. - Tahvilian R, Gravandi MM, Noori T, Papzan A, Jamshidi N, Iranpanah A, Moradi A, Shirooie S. The therapeutic effect of methanolic extract Bryonia dioica Jacq. in a female rat model of polycystic ovary syndrome. Journal of Reports in Pharmaceutical Sciences. 2022 Jan 1;11(1):79-84.
<i>5. Why was a metformin dosage of 20 mg/kg chosen? Is this a standard dose for rat studies? Provide a specific citation for it.</i> Response: added.
<i>6. Please provide supporting evidence from preliminary data or literature for the selection of T. graminifolius extract doses (100 mg/kg and 200 mg/kg).</i> Response: These doses were chosen by following references. The 100mg/kg doses is based on prior studies. While there is no direct literature supporting 200 mg/kg dose, it was chosen to examine dose-dependent effects and is now acknowledged in the limitations. - Kokabian Z, Mirazi N. Study of analgesic effect of Tragopogon graminifolius hydroethanolic extract in male mice. Eur. J. Exp. Biol. 2014;4(4):170-2.
<i>7. Provide the specific dose of sodium pentobarbital used for euthanasia and a reference to support it.</i> Response: Added.
<i>8. Please specify the catalog numbers and suppliers for each ELISA kit.</i> Response: Added.
<i>9. The document mentions using Turkey's multiple comparison procedure. Correct it to Tukey's multiple comparison procedure.</i> Response: The typographical error has been corrected.
<i>10. To enhance clarity and avoid confusion, I recommend using "PCOS group" instead of "untreated group" throughout the manuscript when referring to the group of animals with induced PCOS that did not receive treatment.</i> Response: the term "PCOS group" has been consistently used throughout the manuscript, and the changes have been highlighted.
<i>11. For the sentence, "The findings on testosterone levels are presented in Figure 1. In the untreated group, testosterone levels showed a marked rise," please specify that this increase in testosterone levels in the PCOS group is statistically significant compared to the healthy control group and include the relevant p-value in the text.</i> Response: This sentence has been revised to indicate that the increase in testosterone levels in the PCOS group is statistically significant compared to the healthy control

group, and the corresponding p-value has been added to the text accordingly.
12. <i>Correct the unit for LH levels in Figure 2.</i> Response: Done.
13. <i>If figure legends incorrectly state that the PCOS group received saline via injection, revise them accordingly.</i> Response: All figure legends have been reviewed and corrected for accuracy.
14. <i>When a term is written out in full for the first time, it should be followed by its abbreviation in parentheses. After the first instance, only the abbreviated form should be used. Review the entire manuscript to ensure this rule is followed consistently for all abbreviations.</i> Response: Corrected.
15. <i>The descriptions of the figures are not clear enough. For example, in Figure 1, MET is defined as "Reference group given metformin at a daily dosage of 300 mg/kg by ingestion". The dosage appears to be incorrect because in the methods, the dosage of Metformin is "20 mg/kg body weight each day over a 28-day period." Please ensure that the figure legends are consistent.</i> Response: The dosage inconsistency has been corrected, and all figure legends (Figures 1 to 4) have been revised to ensure clarity and alignment with the method section. The correct dosage of 20 mg/kg has been consistently applied throughout.
16. <i>Please provide more information as to what was observed at the microscopic level to arrive at the end observation. Were there specific scoring systems used to quantify follicular development, cystic changes, or other histological features? In addition, please provide the complete set of the histopathological images with clearer labels in Figure 5.</i> Response: In the H & E test we compared the morphological features of follicles, the presence of cysts. According to the reviewer commend we shown pathological changes in PCOS group with arrow.
17. <i>In the Discussion section, compare your findings with existing literature to provide context. Discuss how your results support or contradict previous studies.</i> Response: The discussion has been revised to include comparisons with prior studies, highlighting where our findings align with or differ from existing literature.
<i>Associate Editor's Comments:</i> <i>Associate Editor 1:</i> <i>Thank you for submitting your manuscript to JJNPP.</i> <i>Please revise the manuscript and submit a point-by-point response to the reviewers' comments and concerns and highlight the changes in the text.</i> <i>I would also greatly appreciate the authors contribution and time for reviewing</i>

manuscripts for this journal. Without the dedication of reviewers like you, it would be impossible to manage an efficient peer review process and maintain the high standards necessary for a successful journal.

OPEN PEER REVIEW

Revision (1)

REFeree : Reviewer

26 May 2025

1. There is an inconsistency in the color and image quality in the PCOS + Extract 100 group with other groups.
2. None of the histological images includes scale bars. Add appropriate scale bars to the image.
3. Your response letter mentioned that arrows were added to indicate pathological changes in the PCOS group. However, no such changes are visible in the current figures. Include arrows to identify relevant structures (e.g., follicles, cysts) in the histological sections.
4. Revise the "Histological Analysis Results" section. Currently, the description is limited to the control group, with no corresponding text describing the histopathological findings in the PCOS group or the treatment groups. Transfer the descriptive content currently embedded in the legend of Figure 5 into the main text of the results section. The figure legend should be concise and include only essential information such as group names, type of staining, scale bar, and magnification.
5. Verify and correct the unit used for testosterone levels. mIU/mL is not appropriate for testosterone, which is typically reported in ng/mL, ng/dL, or nmol/L depending on the assay.
6. In Figure 3, if statistically significant differences exist between the PCOS group and the control group, as well as between the treatment groups and the PCOS group, indicate only these relevant comparisons in the figure. Currently, it appears that some unnecessary comparisons are marked. Furthermore, although the data suggest that FSH levels are reduced in the PCOS group compared to the control group, this important finding is not mentioned in the Results section. Revise the text to include this comparison if it is statistically significant.
7. Specify the dose of sodium pentobarbital.
8. The reference provided for metformin dosing (Farr et al., 2019) does not offer a valid basis for selecting the dose in a PCOS rat model. A more relevant citation from studies using metformin in PCOS rat models is needed to support the chosen dosage.
9. In the Conclusion section, the authors state that "the observed reduction in testosterone and LH levels, along with an increase in FSH, indicates a potential role in

restoring hormonal balance." However, the increase in FSH levels in the treatment groups compared to the PCOS group was not statistically significant. This statement should be revised.

10. While reductions in testosterone and LH were statistically significant, no significant increase in FSH was observed in the treatment groups. The authors are encouraged to discuss this discrepancy.

11. In the Conclusion section, the authors state that "the observed reduction in testosterone and LH levels, along with an increase in FSH, indicates a potential role in restoring hormonal balance." However, the increase in FSH levels in the treatment groups compared to the PCOS group was not statistically significant. This statement should be revised accordingly in both the Conclusion and the Results section of the Abstract. Moreover, the observed changes in blood glucose levels, which were statistically significant and relevant to the metabolic aspect of PCOS, were not mentioned in the Conclusion. It is recommended to include a brief statement summarizing these findings to provide a more comprehensive conclusion.

12. Ensure consistency in unit formatting throughout the manuscript. For example, in the case of "mg/kg" versus "mg per kilogram," the standard and more concise format in scientific writing is typically "mg/kg".

13. Avoid including specific p-values or statistical significance indicators in the Abstract.

14. Revise the title. "Rat" should be changed to "Rats".

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Cover letter

Dear editorial Team of the JJNP journal,
We would like to thank the reviewer and the editorial team for their thoughtful feedback on our manuscript. We have carefully reviewed each comment and have revised the manuscript accordingly. A detailed point-by-point response is included below.

We hope the changes made meet the expectations for acceptance.

Sincerely,
On behalf of Dr. Mohammad Hossein Farzaei
(Corresponding author)

Reviewers' Comments:

Reviewer 1:

1. There is an inconsistency in the color and image quality in the PCOS + Extract 100 group with other groups.

Response: Corrected.

2. None of the histological images includes scale bars. Add appropriate scale bars to the image.

Response: Added.

3. Your response letter mentioned that arrows were added to indicate pathological changes in the PCOS group. However, no such changes are visible in the current figures. Include arrows to identify relevant structures (e.g., follicles, cysts) in the histological sections.

Response: The arrows were added. Long arrow: cysts, short arrows: follicles.

4. Revise the "Histological Analysis Results" section. Currently, the description is limited to the control group, with no corresponding text describing the histopathological findings in the PCOS group or the treatment groups. Transfer the descriptive content currently embedded in the legend of Figure 5 into the main text of the results section. The figure legend should be concise and include only essential information such as group names, type of staining, scale bar, and magnification.

Response: The "Histological Analysis Results" section has been revised to include detailed descriptions of the PCOS and treatment groups, which were previously included in the legend of Figure 5. This ensures that all relevant histopathological findings are presented within the main text of the Results section. Additionally, the legend for Figure 5 has been shortened to include only essential information, such as group names, type of staining, magnification levels, and the scale bar. Specifically, it now notes that the top row shows images at x40 magnification, the bottom row at x100 magnification, scale bar is provided.

5. Verify and correct the unit used for testosterone levels. mIU/mL is not appropriate for testosterone, which is typically reported in ng/mL, ng/dL, or

nmol/L depending on the assay.

Response: We thank the reviewer, the unit has been corrected.

6. In Figure 3, if statistically significant differences exist between the PCOS group and the control group, as well as between the treatment groups and the PCOS group, indicate only these relevant comparisons in the figure. Currently, it appears that some unnecessary comparisons are marked.

Furthermore, although the data suggest that FSH levels are reduced in the PCOS group compared to the control group, this important finding is not mentioned in the Results section. Revise the text to include this comparison if it is statistically significant.

Response: We have revised the Results section to include the statistically significant reduction in FSH levels observed in the PCOS group compared to the control group, as recommended. This key finding is now explicitly stated in the updated text.

Response: the tips were added in the texts.

7. Specify the dose of sodium pentobarbital.

Response: Added.

8. The reference provided for metformin dosing (Farr et al., 2019) does not offer a valid basis for selecting the dose in a PCOS rat model. A more relevant citation from studies using metformin in PCOS rat models is needed to support the chosen dosage.

Response: The reference for metformin dosing has been updated to a more appropriate study that specifically uses metformin in PCOS rat models. This substitution provides a more valid justification for the selected dosage.

9. In the Conclusion section, the authors state that "the observed reduction in testosterone and LH levels, along with an increase in FSH, indicates a potential role in restoring hormonal balance." However, the increase in FSH levels in the treatment groups compared to the PCOS group was not statistically significant. This statement should be revised.

This comment is similar to comment 11.

10. While reductions in testosterone and LH were statistically significant, no significant increase in FSH was observed in the treatment groups. The authors are encouraged to discuss this discrepancy.

Response: This discrepancy is addressed in the Discussion section, where we note the differential responsiveness of gonadotropins to treatment and reference some other studies showing similar hormonal patterns.

11. In the Conclusion section, the authors state that "the observed reduction in testosterone and LH levels, along with an increase in FSH, indicates a potential role in restoring hormonal balance." However, the increase in FSH levels in the treatment groups compared to the PCOS group was not statistically significant.

This statement should be revised accordingly in both the Conclusion and the Results section of the Abstract. Moreover, the observed changes in blood glucose levels, which were statistically significant and relevant to the metabolic aspect of PCOS, were not mentioned in the Conclusion. It is recommended to include a brief statement summarizing these findings to provide a more comprehensive conclusion.

Response: We have revised the relevant sentence in both the Conclusion and the Results section of the abstract to reflect that the increase in FSH was not statistically significant. Additionally, we have included a brief statement in the Conclusion summarizing the observed changes in blood glucose levels to better represent the metabolic findings.

12. Ensure consistency in unit formatting throughout the manuscript. For example, in the case of "mg/kg" versus "mg per kilogram," the standard and more concise format in scientific writing is typically "mg/kg".

Response: Done accordingly.

13. Avoid including specific p-values or statistical significance indicators in the Abstract.

Response: The Results section of the abstract has been revised to remove all specific p-values and references to statistical significance.

14. Revise the title. "Rat" should be changed to "Rats".

Response: Done accordingly.

Associate Editor's Comments:

Associate Editor 1:

The Ms still needs some minor revisions before suitable for publication in JJNPP

EIC Decision:

Our impression is that the above-mentioned manuscript could become suitable for publication in JJNPP. However, the criticism from the referees requires that we defer a final decision until you have considered their remarks and revised your manuscript accordingly.

Uploaded Files by Reviewers/Associate Editor/ or EIC: Below, you can find the list of files attached by reviewers or associate editor or EIC during the review process (if any).

OPEN PEER REVIEW

Revision (2)

REFEREE : Reviewer

10 Jul 2025

Comment:

In Figure 3, treatment groups should be statistically compared only to the PCOS group to properly evaluate treatment effects. Comparing treatment groups directly to the healthy control group is inappropriate.

The PCOS group should be compared to the control group to confirm PCOS induction.

Treatment groups should be compared to the PCOS group to assess treatment efficacy.

Additionally, the statement in the Results section of the abstract regarding the increase in FSH levels should be revised. Since the extract did not significantly increase FSH compared to the PCOS group, the current wording is inaccurate.